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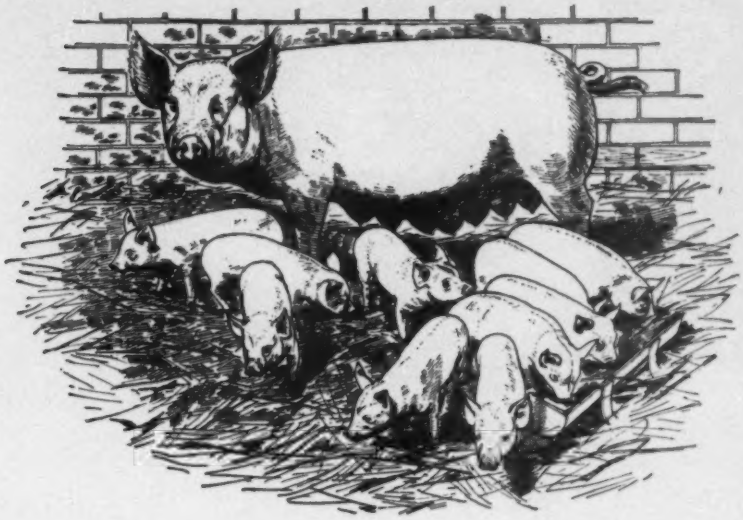

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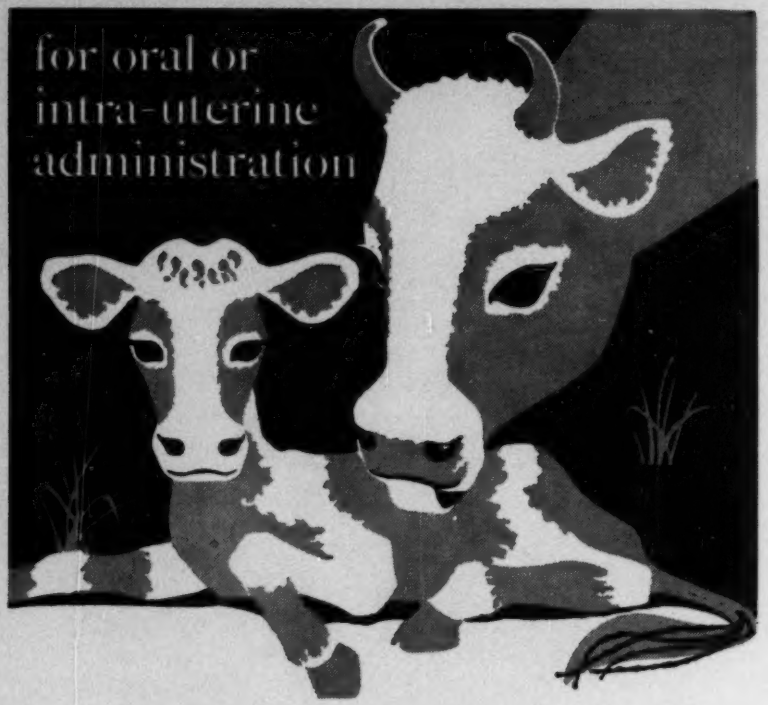

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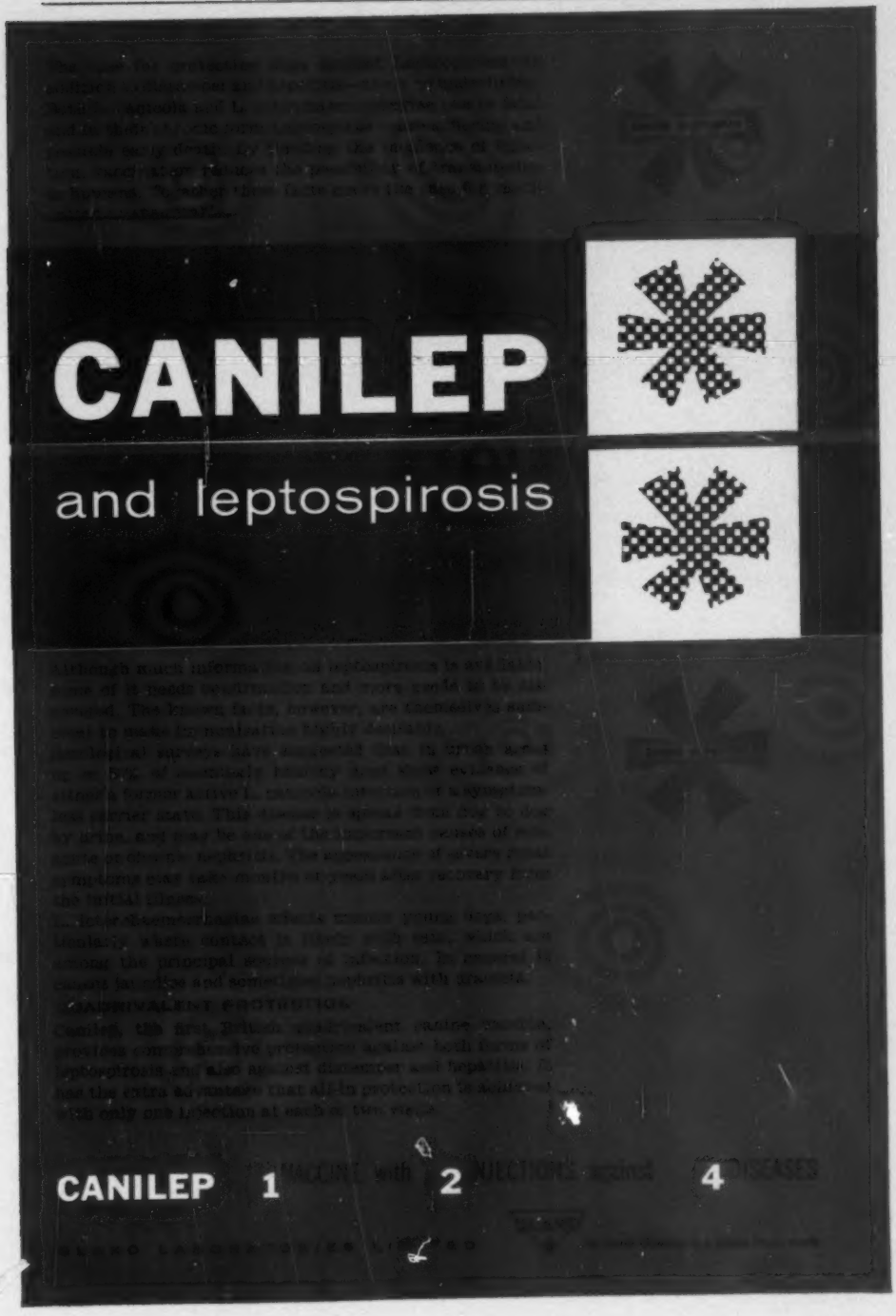
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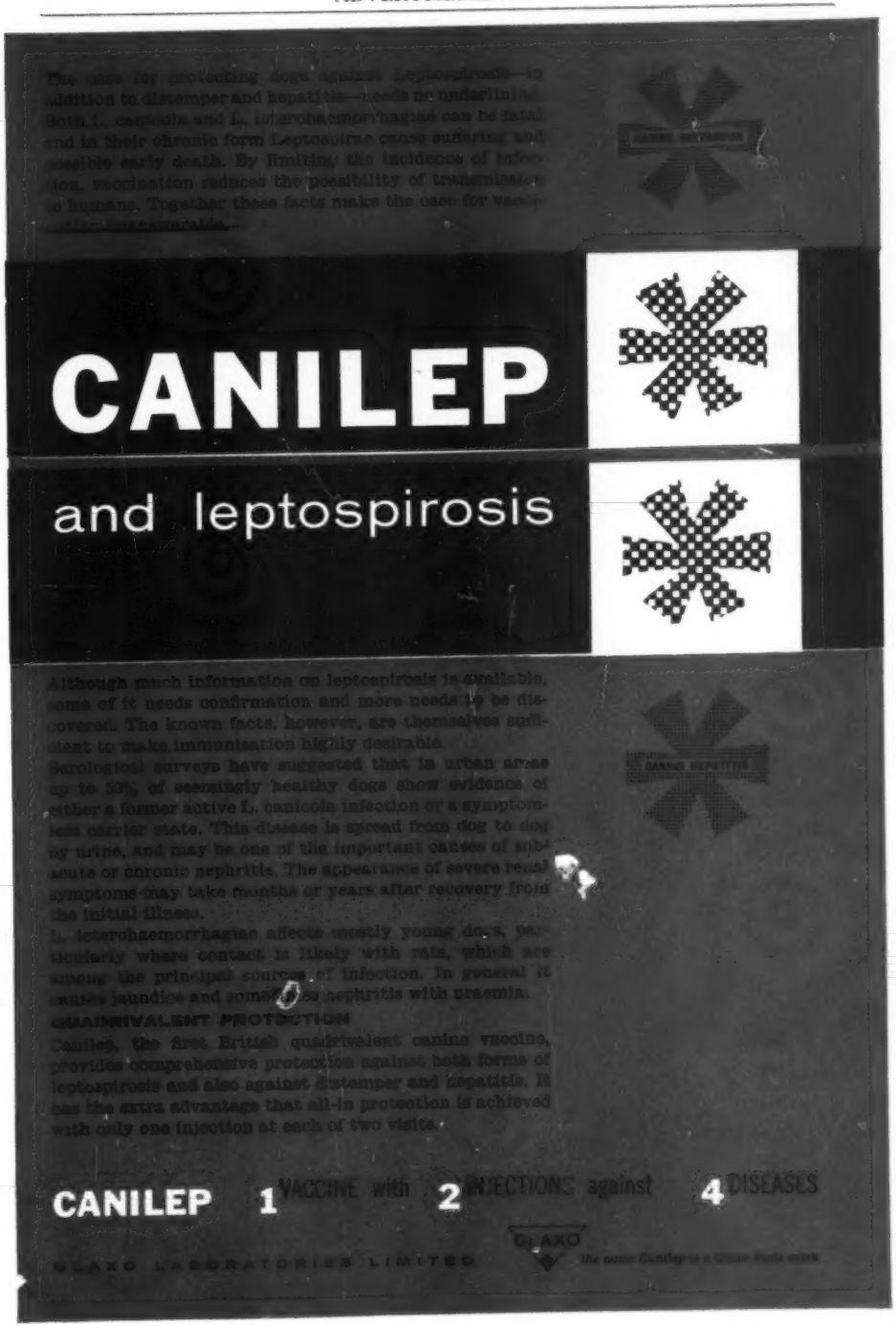
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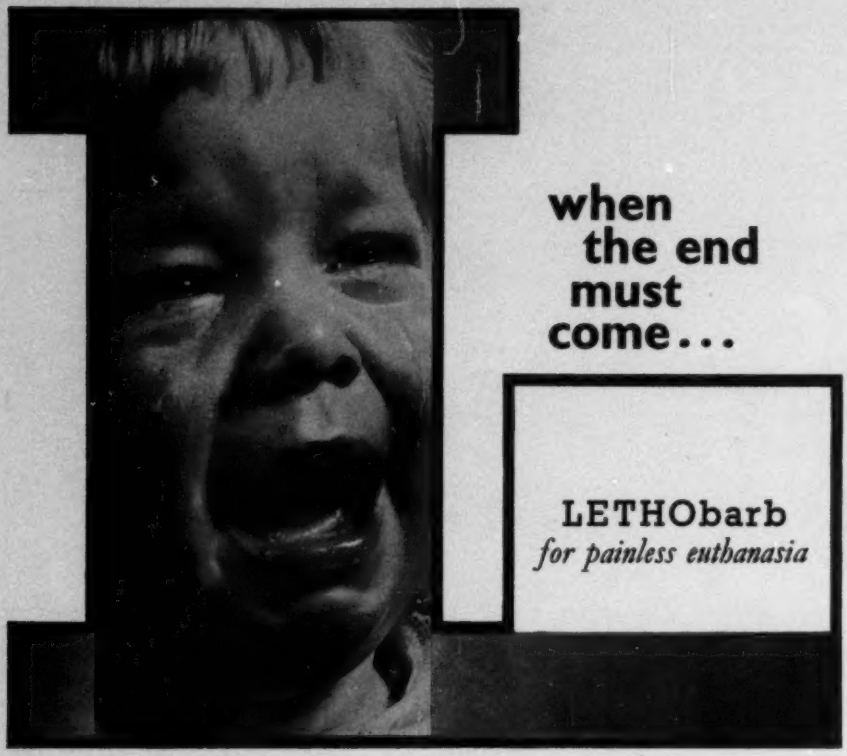

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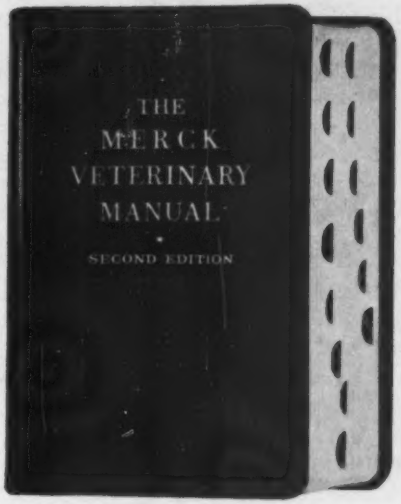

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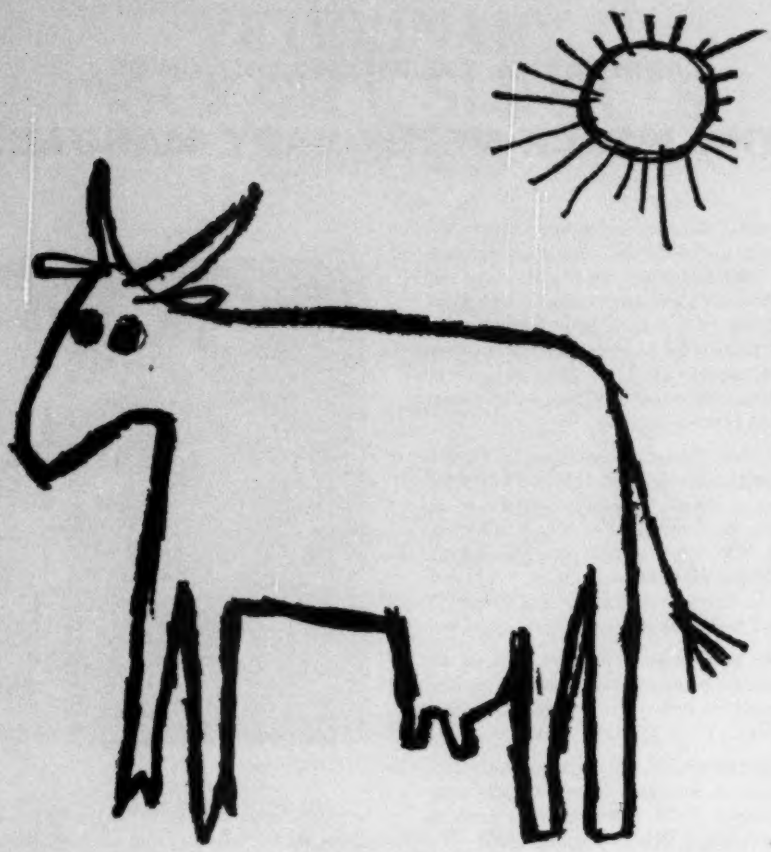

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MEASUREMENT OF CHANGES IN THE BODY TEMPERATURE OF THE OX *(BOS TAURUS)*

By D. L. Ingram* and G. C. Whittow
Department of Physiology, Hannah Dairy Research Institute, Ayr, Scotland

Many studies of thermoregulation in the ox and other animals necessitate the detection of rapid changes in body temperature. Bligh (1957) has produced evidence that a thermocouple chronically implanted in the carotid artery with its temperature-sensitive tip at the bicarotid junction may be used not only to detect such changes, but also to measure the mean temperature of the circulating blood. However, the implanted thermocouple invariably occludes the carotid artery through which it is threaded. This technique cannot be used, therefore, in studies requiring an intact circulation to the brain.

The purpose of the work described here was to compare the temperature measured by a thermocouple in close contact with the outer surface of the carotid artery with the temperature of the blood at the bicarotid junction. Comparisons were made in calves at different steady levels of body temperature and when the body temperature was changing rapidly.

METHODS

Four Ayrshire bull calves, aged from nine to twelve months, were used. Temperatures were measured with 25 S.W.G. copper-constantan thermocouples clad in polythene tubing. The overall accuracy of the temperature-measuring system was ±0·1°C. The temperature of the outer surface of the carotid artery in the neck was measured by soldering the tip of a thermocouple to a ribbon of fine-mesh metallic gauze which was then sewn around the artery. Rectal temperatures were measured by means of 40 S.W.G. thermocouples inserted to a depth of 12–14 cm. In one animal (D) a thermocouple was implanted in the hypothalamus in the manner described by Findlay and Ingram (1961).

The implanted thermocouples were inserted aseptically under Fluothane (2–bromo-2–chloro-1, 1, 1–trifluoroethane; I.C.I.) anaesthesia and their lead wires were attached to perspex contact buttons sewn on the skin of the neck, as described by Bligh (1957). The temperature of the outer surface of the carotid artery was measured on the artery not occluded by the thermocouple used for measuring the temperature of the blood at the bicarotid junction.

The animals were exposed for three to six hours to environmental dry-bulb (DB) temperatures of −5°, 5°, 10°, 15°, 20°, 36°, 40° and 50°C, at low humidity. At environmental temperatures (DB) of 5°, 10°, 20° or 36° two of the animals (A and B) were exposed to infrared irradiation from eight 250 w lamps. This

* Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge.

procedure is known to produce changes of body temperature (Findlay and Ingram, 1961). Increases of body temperature were also produced in two animals (B and C) by feeding them with hay during exposure to environments of 10°, 15° or 20°C. Rapidand large increases in body temperature were produced by exposing one of the animals (C) to an environmental temperature of 40°C, at low humidity, 23°C environmental wet-bulb (WB) temperature, for about one hour and then increasing the humidity until the air was almost completely saturated with water vapour (39°, WB). When the body temperature had reached 42° the humidity, but not the temperature, was reduced. All experiments were conducted in the climatic laboratory described by Findlay, McLean and Bennet (1959).

RESULTS

Table I gives the differences between the temperature of the blood at the bicarotid junction and that of the outer surface of the carotid artery. Six hundred and thirty estimations of the difference of temperatures were made.

TABLE I

DIFFERENCES BETWEEN THE TEMPERATURE OF THE BLOOD AT THE BICAROTID JUNCTION AND THE TEMPERATURE ON THE SURFACE OF THE CAROTID ARTERY, IN °C, WHEN THE BODY TEMPERATURE CHANGED AS A RESULT OF EXPOSURE TO INFRARED IRRADIATION (I.R.), FEEDING (F), EXPOSURE AT HIGH ENVIRONMENTAL HUMIDITY (H.) AND EXPOSURE AT DIFFERENT ENVIRONMENTAL TEMPERATURES

Animal	*Environmental conditions**	*Number of experiments*	*Number of estimations of the temperature difference † with a magnitude of:* +0·2	+0·1	0·0	−0·1	−0·2	−0·3
A	10° I.R.	1	–	–	9	45	6	–
	20° I.R.	1	1	6	15	10	–	–
	36° I.R.	2	7	20	35	2	–	–
B	5° I.R.	2	–	2	68	27	6	–
	20° I.R.	2	–	7	43	41	3	–
	36° I.R.	1	1	8	15	15	8	–
	20° F.	1	–	–	15	17	5	–
C	−5° ‡	1	–	–	1	6	22	4
	30° ‡	1	–	–	9	9	–	–
	40° H.	1	2	9	20	14	–	–
	10° F.	1	–	–	–	19	24	4
	15° F.	1	–	–	9	23	17	1
		Total	11	52	239	228	91	9

* Environmental dry-bulb temperatures.

† A positive temperature difference means that the temperature on the surface of the carotid artery was higher than that of blood at the bicarotid junction.

‡ At these environmental temperatures animal C was not exposed to infrared irradiation or to a high humidity, nor was it fed.

In only nine instances was the temperature difference outside the limits of accuracy of the temperature measuring system, i.e. $>0{\cdot}2$°C. In these nine instances the temperature on the surface of the carotid artery was lower than the temperature of the blood at the bicarotid junction.

The changes in body temperature which occurred as a result of feeding, infrared irradiation and exposure of the animals to different environmental temperatures and humidities are given in Table II. The greatest and most

TABLE II

MAXIMAL CHANGES OF THE TEMPERATURE OF THE BLOOD AT THE BICAROTID JUNCTION INDUCED BY THE VARIOUS EXPERIMENTAL PROCEDURES

Animal	*Experiment**	*Range of body temperature* (°C)			
A	10° I.R.	(38·4	— 38·8)	=	0·4°
	20° I.R.	(38·6	— 38·4)	=	0·2°
	36° I.R.	(39·5	— 40·8)	=	1·3°
B	5° I.R.	(38·7	— 39·3)	=	0·6°
	20° I.R.	(38·5	— 39·4)	=	0·9°
	36° I.R.	(39·0	— 41·6)	=	2·6°
	20° F.	(38·4	— 38·8)	=	0·4°
C	−5°	(38·6	— 39·1)	=	0·5°
	5°	(39·1	— 39·5)	=	0·4°
	40° H.	(38·4	— 41·9)	=	3·5°
	10° F.	(38·2	— 38·8)	=	0·6°
	15° F.	(38·2	— 39·0)	=	0·8°

* Notation as in Table I.

rapid changes of body temperature were produced by infrared irradiation or a change in the humidity of the air at high environmental temperatures.

Changes in the temperature of the blood at the bicarotid junction and in the temperature on the surface of the carotid artery in animal C when the humidity of the air was changed at an environmental temperature of 40°C are illustrated in Fig. 1. The difference in temperature between the blood at the bicarotid junction and the outer surface of the carotid artery was always well within the limits of the temperature-measuring system (±0·1°C). The rectal temperature was higher than the temperature of the blood at the bicarotid junction by 0·3° before the humidity was increased. During the period of high humidity the rectal temperature exceeded the temperature of the blood at the bicarotid junction by 0·0 – 0·3°. When the humidity was decreased the rectal temperature continued to increase and did not begin to decrease until 25 minutes after the humidity was reduced. Twenty-five minutes after the humidity was reduced the rectal temperature exceeded both the temperature of the blood at the bicarotid junction and the temperature on the surface of the carotid artery by 0·7°.

In most experiments, the temperature within the rectum exceeded that of the blood at the bicarotid junction by as much as 0·6°. At environmental temperatures of 5° and 10°, however, the rectal temperature was less than both the temperature of the blood at the bicarotid junction and the temperature on the surface of the carotid artery.

The temperature of the hypothalamus, in animal D, together with the temperature of the blood at the bicarotid junction, was measured before, during and after infrared irradiation of the animal in an environment of 10°. The results of this experiment are recorded in Fig. 2. The temperature of the

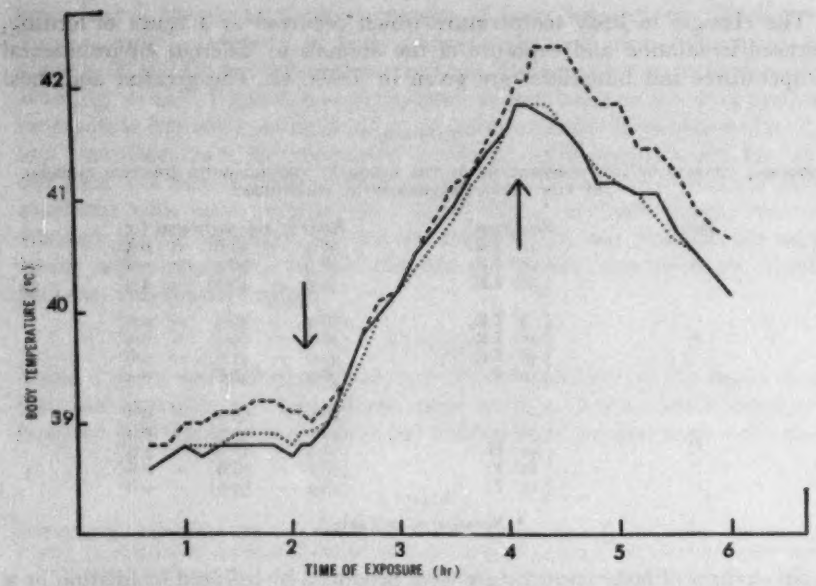

FIG. 1. Animal C. Relationship between the temperature of the blood at the bicarotid junction (. . .), on the surface of the carotid artery (——) and in the rectum (- - -) during changes of environmental humidity. The humidity was high during the period between the arrows. The environmental dry-bulb temperature was 40°C throughout the experiment.

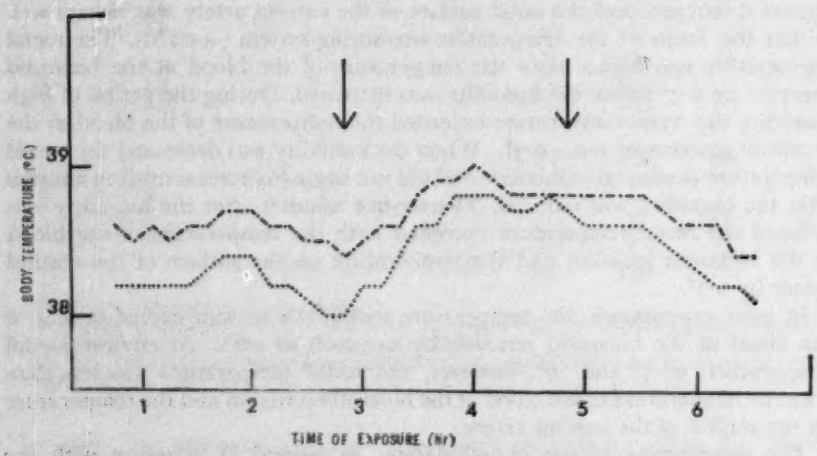

FIG. 2. Animal D. Relationship between the temperature of the blood at the bicarotid junction (. . .) and of the hypothalamus (- . - .) during infrared irradiation of the animal's trunk. The infrared lights were switched on for the period of time between the arrows. Environmental dry-bulb temperature, 10°C.

hypothalamus was 0·5° higher than the temperature of the blood at the bicarotid junction before infrared irradiation. After infrared irradiation of the animal for approximately one hour the temperature of the hypothalamus had increased by 0·5° while the temperature of the blood at the bicarotid junction had increased by 0·8°. These findings conform with those published by Findlay and Ingram (1961) on the relationship between the temperature of the blood at the bicarotid junction and the temperature of the brain.

DISCUSSION

The temperature of the blood at the bicarotid junction provides the best single estimate of the mean temperature of the circulating blood (Bligh, 1957). In the present investigation the temperature of the outer surface of an unoccluded carotid artery was, in almost all conditions, identical with the mean temperature of the blood, within the limits of accuracy (±0·1°C) of the temperature-measuring system used. When the temperatures of these two sites differed by more than 0·2°C, at environmental temperatures of −5°, 10° and 15°, the temperature on the surface of the carotid artery was always less than that of the blood at the bicarotid junction. This was to be expected because the thermocouple on the carotid artery was closer to the skin surface of the animal than the thermocouple which lay in the blood stream and because the temperature of the skin surface at environmental temperatures of −5°, 10° and 15° would be less than the temperature of the blood at the bicarotid junction.

Measurement of the temperature of the outer surface of the carotid artery has therefore all the advantages of the technique developed by Bligh (1957) for measuring the mean temperature of the circulating blood, with the additional advantage of not interfering with the blood supply to the brain and other tissues of the head.

Comparisons between the temperature of the blood at the bicarotid junction and that within the rectum of the ox have been made by Bligh (1957) at environmental temperatures of 20°, 30°, 35° and 40°. He found that the rectal temperature exceeded the temperature of the blood at the bicarotid junction by approximately 0·3°C. The results of the present investigation confirm Bligh's findings for these environmental temperatures and they provide the additional information that, at environmental temperatures of 5° and 10°, the rectal temperature may be lower than the temperature of the blood at the bicarotid junction. At environmental temperatures of 5° and 10° cooling of the blood at the periphery is probably minimal.

At an environmental temperature of −5°, however, the rectal temperature again exceeded the temperature of the blood at the bicarotid junction. At an environmental temperature of −5° the skin temperatures of the ears of calves increased periodically and these increases of ear temperature are followed by cooling of the blood (Ingram and Whittow, to be published). Shivering also occurs at an environmental temperature of −5° and is first observed in the femoral region (Whittow, 1961). Warm venous blood from this part of the body could therefore cause an increase of rectal temperature.

In animal D the temperatures of the hypothalamus and of the blood at the bicarotid junction both increased during infrared irradiation but the increase in the temperature of the blood tended to be greater than that of the hypothalamus. Similar results were obtained by Findlay and Ingram (1961). Consequently, if precise information is required about temperature thresholds of the thermoregulatory centres in the hypothalamus, the temperature-measuring device must be placed in the hypothalamus itself rather than in the blood supply to the brain.

SUMMARY

A technique is described for measuring the temperature of the outer surface of the carotid artery. This technique can be used to measure the mean temperature of the circulating blood to within ±0·1°C under a variety of conditions which result in changes of the body temperature. This method has the additional advantage that it does not interrupt the blood supply to the brain.

ACKNOWLEDGEMENTS

The authors thank Dr. J. D. Findlay for his helpful advice and criticism, and Miss D. McLelland and Miss M. Drysdale for their skilled technical assistance. The work was done in a climatic laboratory built with the aid of a grant from the Colonial Development and Welfare Fund.

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FURTHER OBSERVATIONS ON HELMINTHIASIS IN NIGERIAN ZEBU CATTLE: NATURAL AND ACQUIRED RESISTANCE

By J. G. Ross*, J. Armour† and R. P. Lee‡

Department of Federal Veterinary Research, Vom, Nigeria

Ross, Lee & Armour (1959), Ross, Armour & Lee (1960), and Ross & Armour (1960) outlined the changes in serum albumin and γ-globulin levels and the immunological response shown by the complement fixation test in natural strongyle infestations of cattle. Evidence was presented to suggest the occurrence of lines of cattle genetically resistant or susceptible to infestation with these parasites, and to indicate that resistance was associated with immune response. The present investigation was primarily designed to obtain further information on pathogenicity of haemonchosis but it was also used to study the immunological response and changes in the serum protein fraction concentrations.

DESIGN OF EXPERIMENT

Ten calves, White Fulani Zebu and White Fulani Zebu/Friesian crosses from the Livestock Investigation Centre, Vom, were faecal-sampled fortnightly from the beginning of April, 1958, until mid-November of that year. All calves were bucket-fed with whole milk, supplemented by hay and concentrates, until mid-June when they were weaned at between four and six months of age.

The ten calves were divided into two groups of five. Group 1 was reared parasite-free on concrete from birth until early July. Group 2 was reared in the farm calf shed and was consequently subject to a low strongyle challenge from birth. Early in July both groups were placed together in a small paddock. All the animals were dosed with infected larvae (60 per cent *Haemonchus* spp.) at the rate of 1,500 larvae daily for five days, followed four weeks later by doses of 15,000 larvae. Heavy strongyle burdens rapidly developed in both groups which soon became subject to a heavy natural challenge as the artificially induced infection developed and contaminated the pasture. The treatments were thus as follows:

Group 1: Parasite-free, birth to June. Heavy infection July onwards.
Group 2: Low infection, birth to June. Heavy infection July onwards.

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In animal D the temperatures of the hypothalamus and of the blood at the bicarotid junction both increased during infrared irradiation but the increase in the temperature of the blood tended to be greater than that of the hypothalamus. Similar results were obtained by Findlay and Ingram (1961). Consequently, if precise information is required about temperature thresholds of the thermoregulatory centres in the hypothalamus, the temperature-measuring device must be placed in the hypothalamus itself rather than in the blood supply to the brain.

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TABLE I

RESULTS OF STRONGYLE FAECAL EGG COUNT EXAMINATIONS WITH TWO GROUPS AND FOUR INDIVIDUAL CALVES

Observation date	16.6.58	1.7.58	15.7.58	29.7.58	11.8.58	27.8.58	8.9.58	22.9.58	6.10.58	20.10.58	3.11.58	17.11.58
Group 1 Mean E.P.G.*	Neg.	Neg.	Neg.	4	1,558	3,964	6,330	7,238	6,216	7,200	4,525	1,205
Group 2 Mean E.P.G.	270	410	324	636	1,290	1,884	2,254	1,710	1,342	1,320	1,016	542
Group 1 E.P.G. resistant calf no. 3531	Neg.	Neg.	Neg.	10	1,150	2,570	3,450	2,960	6,690	1,420	1,010	350
Group 1 E.P.G. susceptible calf no. 3556	Neg.	Neg.	Neg.	Neg.	2,270	3,090	9,170	Died	—	—	—	—
Group 2 E.P.G. resistant calf no. 3555	450	500	220	490	2,330	1,250	30	60	160	580	260	150
Group 2 E.P.G. susceptible calf no. 3549	Neg.	160	180	830	1,360	3,570	8,080	3,370	4,260	3,850	3,490	1,750

* Eggs per gram.

MATERIALS AND METHODS

Strongyle faecal egg counts were carried out fortnightly on individual calf samples, following the technique of Roberts & O'Sullivan (1950). Blood was collected from the jugular vein of all animals monthly, allowed to clot and the serum separated and stored at —20°C. Serum protein fraction estimates were made by paper electrophoresis techniques, and complement fixation tests were carried out on bulked group and individual sera, as indicated later in the paper. The methods of bulking sera and the above serological tests have been described previously (Ross *et al.*, 1959, 1960). One minor change in antigen preparation for the complement fixation test was introduced. A mixed larval antigen consisting of *Haemonchus* spp., 19 per cent, *Cooperia* spp., 57 per cent, *Oesophagostomum* spp., 20 per cent, and *Trichostrongylus* spp., 4 per cent, was used in place of the whole worm antigen. Both Stewart (1950) and Soulsby (1956) have demonstrated cross-reactions between antigens and antibodies of different nematode species; consequently a mixed antigen would seem more suitable to demonstrate antibody production, where mixed infections occur, than individual antigens. Antigen preparation followed the method of Stewart (1948), except that larvae were not weighed but concentrations of 40,000 larvae/ml. were used in the initial stages. The heated antigen was freshly prepared every seven days and was normally potent at dilutions between 25 and 12 per cent. Potencies of serum samples are expressed as "index ratios", that is, the ratio of the 50 per cent haemolysis end-point of the complement in the presence of the test serum and antigen, to that with the test serum only (Rice, 1942; Ross *et al.*, 1960).

RESULTS

The strongyle infection which developed consisted principally of *Haemonchus* spp. and *Cooperia* spp. A low *Oesophagostomum* spp. infection developed between August and November. Undifferentiated total strongyle faecal egg counts only are considered here.

The groups' mean faecal strongyle egg counts and the bulk serum complement fixation index ratios and serum protein fraction levels are shown in Tables I and II.

Comparison of strongyle faecal egg counts in groups 1 and 2 demonstrates the ability of cattle to develop a resistance to subsequent heavy challenge infections following a preliminary mild infection. This acquired resistance does not appear to be associated with a parallel increase in the rise in antibody level. In group 1, three out of the five calves died in August and September from helminthiasis. This demonstrates the pathogenic nature of the heavy challenge infection in previously uninfected calves and the resistance acquired by the calves in group 2, where no deaths occurred. After the deaths of the three susceptible calves in group 1 the mean strongyle faecal egg counts based on the two surviving resistant calves were considerably reduced, from September onwards, and the mean complement fixation index ratios over the same period were higher.

Apart from group differences, marked differences were evident in the level of infection and serological response between individuals in the groups. These

differences were most marked in group 1, where three calves died and two survived, and to a lesser degree in group 2 which also acquired a heavy infection. One resistant and one susceptible calf in group 1 and two similar calves in group 2 were examined individually. The course of the faecal strongyle egg counts and serological changes in these calves are shown in Tables I and III.

TABLE II

MEAN SEROLOGICAL RESULTS IN TWO GROUPS

	Observation date	16.6.58	1.7.58	11.8.58	8.9.58	20.10.58	17.11.58
	Complement fixation index ratio	16	11	31	16	21	41
Group 1 (Means)	Albumin (g./100 ml.)	2·57	2·20	2·22	1·67	1·57	1·82
	γ_1–Globulin (g./100 ml.)	0·67	0·40	0·38	0·68	0·83	0·81
	γ_2–Globulin	0·36	0·46	0·96	0·67	0·70	1·31
	Complement fixation index ratio	15	11	21	15	21	31
Group 2 (Means)	Albumin (g./100 ml.)	2·60	2·40	2·08	1·96	1·47	1·86
	γ_1–Globulin (g./100 ml.)	0·62	0·43	0·67	0·66	0·95	0·97
	γ_2–Globulin (g./100 ml.)	0·66	0·78	0·93	0·87	1·05	0·63

TABLE III

COMPLEMENT FIXATION, INDEX RATIOS AND SERUM ALBUMIN LEVELS IN FOUR INDIVIDUAL CALVES

	Observation date	1.7.58	11.8.58	8.9.58	20.10.58	17.11.58
Resistant calf No. 3531 group 1	Complement fixation index ratio	1	6	10	60	23
	Albumin (g./100 ml.)	2·69	3·06	2·53	1·41	1·78
Susceptible calf No. 3556 group 1	Complement fixation index ratio	6	7	7	Died	—
	Albumin (g./100 ml.)	3·35	1·67	1·15	Died	—
Resistant calf No. 3555 group 2	Complement fixation index ratio	1	16	11	16	31
	Albumin (g./100 ml.)	2·15	1·93	1·80	1·42	1·68
Susceptible calf No. 3549 group 2	Complement fixation index ratio	1	3	3	7	7
	Albumin (g./100 ml.)	2·08	2·87	1·70	2·02	1·27

Compared to the resistant calves, the susceptible calves show higher strongyle faecal egg counts and lower complement fixation antibody levels. These differences confirm similar findings in a genetically resistant line of calves previously described (Ross *et al.*, 1960).

No significant differences were evident between the various serum globulin fraction levels in the resistant and susceptible calves. No additional support is given to the previously observed association between complement fixation antibody levels and strongyle faecal egg counts, and γ_1- and γ_2-globulin, respectively. More recent work has, however, shown that the complement fixing antibody is principally located in the γ_1-globulin fraction (Ross, 1961). These findings agree with those of Stewart (1950) that the complement fixing antibody levels do not give a measure of acquired resistance; they do suggest, however, that the levels give a measure of an animal's inherent resistance, in support of previous findings (Ross *et al.*, 1960).

DISCUSSION

In a previous study of genetical resistance to strongyle worm infestations in Zebu calves (Ross *et al.*, 1960) resistant calves were shown to exhibit low strongyle faecal egg counts and high complement fixation index ratios, whereas susceptible calves had high strongyle faecal egg counts and low complement fixation index ratios. The overall seasonal changes in the faecal egg counts and complement fixation index ratios in the resistant and susceptible calves did not, however, demonstrate the inverse relationship reported by Stewart (1950) in sheep infections. It was thought that this may have been obscured by the relatively wide spacing of the serological examinations. In the current investigation the inverse relationship of faecal egg counts and complement fixation index ratios was evident in both groups.

When the complement fixation index ratios of the two groups and the vastly differing pattern of infestations experienced by the groups are considered, several points of interest emerge. Except where individuals with innate resistance predominate, as occurred in group 1 after the death of three susceptible calves, the level of the complement fixation index ratio is not a measure of an acquired resistance or of the level of infection occurring. Previous work (Ross *et al.*, 1960) has shown that innate resistance to strongyle infestations in cattle is associated with a high antibody response. This is further substantiated by the results shown in Table III for the two resistant and two susceptible calves from groups 1 and 2. In the susceptible calves the complement fixation index ratios never rose above ten despite a very high strongyle infection. In the susceptible calf No. 3549 in group 2, the rise in antibody level after the reduction in faecal egg count in September was small. In the two resistant calves with low strongyle infections the picture is that of a more or less steady rise in antibody level with a marked increase in October concurrent with the striking drop in faecal egg counts.

Serum albumin levels were accurate guides to condition and proved of considerable value in assessing the pathogenic effect of the infections in the groups. As in previous investigations (Ross *et al.*, 1959, 1960), the resistant

calves which showed low faecal egg counts, high complement fixation index ratios and maintained above-average serum albumin levels, made above-average weight gains. A detailed assessment of the correlation between faecal egg counts, serum albumin levels and weight gains in individual calves has been published elsewhere (Ross & Armour, 1960). This and previous work suggests that the occurrence of resistant calves in a random sample may be up to 25 per cent. The common occurrence of these calves, coupled with the association between resistance and an increased immunological response in strongyle infections, provides a useful tool in further studies into the mechanism of the resistance.

SUMMARY

The immunological response measured by the complement fixation test and serological changes estimated by electrophoresis of serum proteins are compared in varying strongyle infections in Zebu cattle. The occurrence of acquired and innate resistance is demonstrated. The latter is shown to be associated with increased complement fixing antibody production, whereas the former is unrelated to production of this antibody. Differences between the varying infection groups and between resistant and susceptible calves are discussed.

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POTASSIUM CHLORATE AS A PROPHYLACTIC TREATMENT FOR SUBCLINICAL KETOSIS

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Clinical ketosis in dairy cattle is well known to cause considerable financial loss because it results in the cows being in poor condition and lowers their milk yields. Subclinical ketosis also can depress milk yield (Blackburn, Castle & Strachan, 1958; Blackburn, Castle, Drysdale & Strachan, 1961; Holmes, 1958; Penny, 1958), and it may well cause losses equal to those that result from clinical ketosis because of the difficulty of detecting it under normal conditions of farm management. If, therefore, conditions are such that subclinical ketosis is likely to occur a prophylactic treatment could be extremely valuable in avoiding the loss of milk yield that accompanies this disease.

Quite recently it was shown by Good (1958) and by Blackburn, Castle & Drysdale (1959) that in clinical and subclinical ketosis potassium chlorate given orally greatly reduced the ketone content of the blood and milk and increased the milk yield to the value that might have been expected had ketosis not occurred. This valuable therapeutic effect of potassium chlorate was so marked that it was decided to conduct the present experiment to study the value of potassium chlorate when fed as a prophylactic agent. The results of this work show that this method of feeding the chlorate over a period during which ketosis might be expected to occur was not effective in preventing the disease or the decrease in milk yield which it causes.

EXPERIMENTAL

Feeding and management

Fourteen Ayrshire cows which had calved during February and March, 1960, were fed a ration of 60 lb. grass silage and 10 lb. dried grass per day. Before calving a "steaming-up" ration of home-mixed concentrates was fed at a rate of 2 lb./cow/day in the fourth week before calving and this rate was increased until 6 lb./cow/day was fed in the week immediately before calving. The total amount fed was approximately 130 lb./cow. After calving the concentrate ration was increased gradually to 14 lb./cow/day and remained at this level until the fifteenth day when the rate of feeding became 4 lb./10 lb. milk for all milk yields over 5 lb./day.

At calving the cows were allocated to one of two treatments. Cows on the control treatment were fed the ration of silage, dried grass and concentrates,

whereas those on the prophylactic treatment were fed the same ration as the control cows but, in addition, from the evening of the sixth to the morning of the eighteenth day after calving 1 oz. potassium chlorate per day was mixed with the concentrate ration. Each cow on the prophylactic treatment received a total of 12 oz. of potassium chlorate in the 12-day feeding period.

The cows were housed in a normal byre during the experiment but were penned in a bare paddock for two hours each day.

Records

Milk yields were recorded twice daily at milking times. Samples of evening milk from each cow were examined each day by Rothera's test, and, according to the colour intensity, the samples were graded as negative, or +1, +2, +3 or +4. Samples of blood were taken at weekly intervals from each cow from four weeks before the cows calved until they went to grass, and the total concentration of acetone bodies in the blood was determined by the method of Thin & Robertson (1952).

Treatment of cows

Originally it had been decided not to treat any cows which developed ketosis in the two groups for the first 36 days after calving, but owing to the severity of the ketosis in one cow in the control group, treatment was considered necessary. Potassium chlorate at a rate of 1 oz. twice daily for three days was fed to this cow and the treatment cured it. No other cows were treated in this first 36-day period, but thereafter any animals which showed signs of severe ketosis with a rapid decline in milk yield were treated by this same method. Three cows in each group required treatment.

RESULTS

Feed consumption

The silage and dried grass were eaten regularly, but there were some sporadic refusals of concentrates when ketosis developed. Because of these refusals it was necessary to administer the chlorate in a paper bolus for three consecutive days to two cows on the prophylactic treatment. Thereafter no further trouble was experienced and all cows in this group received the intended total quantity of 12 oz. of potassium chlorate.

Rothera's tests

Results of the daily Rothera's tests during the 36-day experimental period are shown in Table I. In the first 18 days when the potassium chlorate was fed, the milder form of ketosis, as indicated by the +1 and +2 gradings, tended to be more prevalent in the control group, whereas of the samples giving readings of +3 and +4 ten were in the control group and six in the group receiving potassium chlorate. The control group had 19 more positive reactions than the prophylactic group. In the subsequent period between the 18th and 36th day of lactation this position was reversed and there were 29 more positive

TABLE I

THE NUMBER OF SAMPLES OF EVENING MILK ON THE CONTROL AND PROPHYLACTIC TREATMENTS GRADED AS A RESULT OF ROTHERA'S TEST

Grade of Rothera's test reaction in the milk	*Days after calving* 1–18		19–36	
	Control	*Prophylactic*	*Control*	*Prophylactic*
+1	27	18	32	24
+2	20	14	26	29
+3	8	5	8	30
+4	2	1	0	12
Total positive samples	57	38	66	95
Total negative samples	69	88	60	31

samples in the prophylactic group than in the control group. The total numbers of +1 and +2 reactions in this period were 58 and 53 for the control and prophylactic groups respectively, the corresponding totals for the +3 and +4 reactions being 8 and 42. In the two periods together there were 133 positive samples in the treated group and 123 in the control group.

Fig. 1 shows the number of cows in both groups in which ketosis developed, as determined by positive Rothera's tests. During the prophylactic feeding of

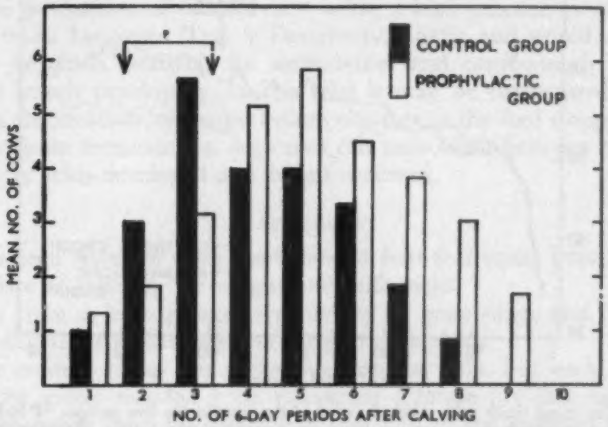

Fig. 1. The number of cows from which milk gave positive Rothera's test grouped in periods of 6 days after calving. P indicates the period when potassium chlorate was fed to the prophylactic group.

chlorate there were more animals with ketosis in the control group than in the prophylactic group, but after 18 days this position was changed and a noticeably higher incidence of the disease was found in the prophylactic group.

Blood ketones

The weekly values for the ketone content of the blood are given in Table II. Before calving there was little difference between the mean values of the two

TABLE II

KETONE BODIES IN THE BLOOD OF THE COWS ON THE CONTROL AND PROPHYLACTIC TREATMENTS

Group	*Days before calving*				*Days after calving*				
	28–22	21–15	14–8	7–0	0–7	8–14	15–21	22–28	29–35
	mg. acetone/100 ml.								
Control	9·5	6·6	8·3	5·6	8·4	14·0	16·2	16·2	21·1
Prophylactic	10·6	6·1	8·7	6·4	12·8	13·7	21·0	21·5	28·6

(The values are the means for the seven cows in each group)

groups but after calving the prophylactic group had slightly higher values than the control group. These higher values in the prophylactic group were recorded in the first few days after calving before the chlorate was fed and between the 18th and the 36th day after calving.

Milk yield

The mean daily milk yields per cow for the first 60 days of lactation are illustrated in Fig. 2. The lactation curves of both groups followed a very

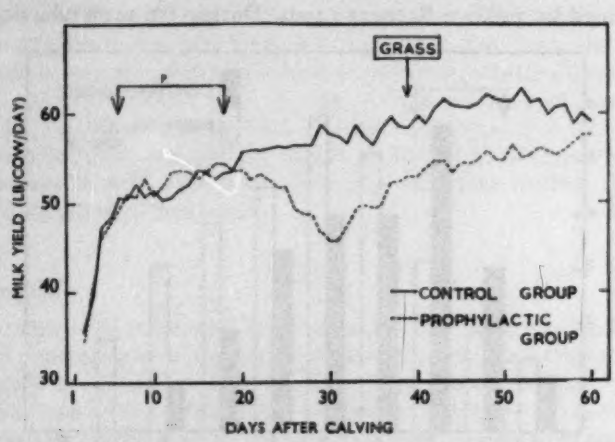

Fig. 2. The mean daily milk yield (lb./cow) of the cows in the two groups. P indicates the period when potassium chlorate was fed to the prophylactic group. Grazing started on average 39 days after calving.

similar pattern until the 18th day when the feeding of the chlorate stopped. Thereafter the prophylactic group declined in milk yield and did not equal that of the control group until 60 days after calving.

Subsequent health

No harmful effects of the potassium chlorate feeding were recorded and the mean lactation yield of the seven cows on the prophylactic treatment was 981 gallons.

DISCUSSION

In this trial the prophylactic feeding of potassium chlorate appeared at first to have a small effect in reducing the level of subclinical ketosis without altering milk yields, but when the feeding of the chlorate stopped on the morning of the 18th day after calving the prophylactic group showed a marked increase in ketosis. This was a severe type and was accompanied by a large decrease in milk yield (Fig. 2). The overall effect was that the prophylactic feeding treatment reduced the total milk yield in both the 36 and the 60-day periods after calving, in contrast to the increase in yield which was found in earlier work with a short-term feeding period of three days (Blackburn, Castle & Drysdale, 1959). Since this three-day treatment was again effective when used to cure ketosis in this trial, the increased length of the experimental feeding period, 12 days instead of three, must in some way have affected the beneficial effect of the chlorate.

There can be no doubt that it is the chlorate and not the potassium ion which is the active constituent against ketosis (Talsma, 1952), and by analogy with another oxidizing agent, magnesium peroxide used by Skamser & Proewig (1958), it is thought that potassium chlorate may act in a similar way by stopping or slowing down temporarily the fermentation by anaerobic bacteria of sugars, starches and cellulose in the rumen. This action would reduce the production of volatile fatty acids, a high percentage of which are reported to be ketogenic (Dye & Dougherty, 1956), and would allow more sugars to be made available for assimilation and consequently reduce the chance of ketosis developing. In this trial it may be conjectured, therefore, that when the inclusion of the potassium chlorate in the feed stopped, the rate of carbohydrate fermentation increased, an imbalance between glucose and volatile fatty acids developed and ketosis occurred.

SUMMARY

(1) Fourteen Ayrshire cows were divided into two equal groups balanced for age, date of calving, live weight and milk yield.

(2) The cows in both groups were fed 60 lb. grass silage and 10 lb. dried grass per day and concentrates according to milk yield.

(3) The control group was given these rations only, but each cow in the prophylactic group was fed 1 oz. potassium chlorate per day mixed in the concentrate ration from the evening of the sixth to the morning of the 18th day after calving.

(4) In the first 18 days of lactation the prophylactic treatment had a negligible effect in reducing the level of subclinical ketosis compared with the control group.

(5) After the 19th day in the prophylactic group, when the feeding of the potassium chlorate stopped, the cows developed a more severe type of ketosis than previously and milk yields declined rapidly.

(6) It is concluded that although potassium chlorate can be used successfully as a therapeutic agent against ketosis it is unsuitable as a prophylactic when fed as described in this trial.

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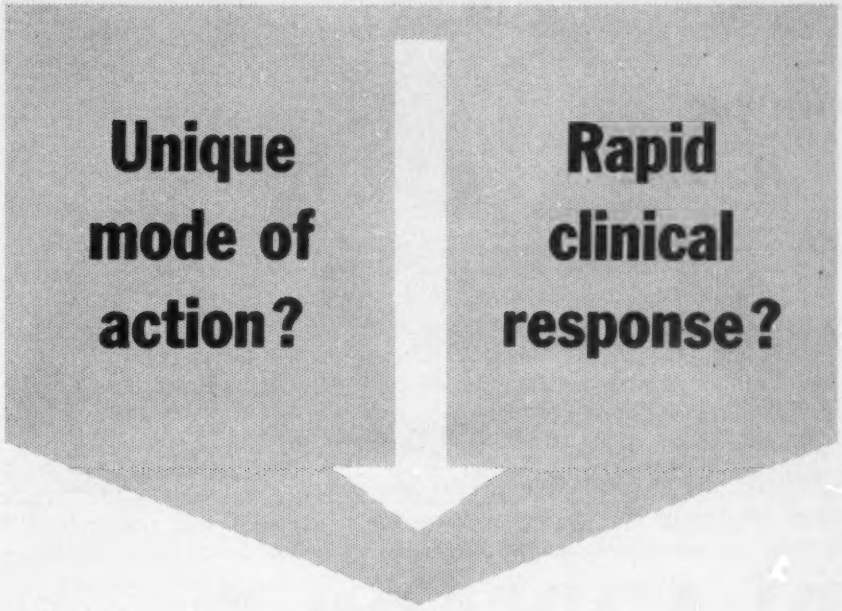

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Brit. vet. J. (1961), 117, 497

A FURTHER REPORT ON ETROLENE AND WARBLE INFESTATION IN CATTLE

By J. K. L. Pearson and J. T. Baxter

The Veterinary Research Division, The Farm, Stormont, and Department of Clinical Veterinary Practice, Trinity College, Dublin

In recent years attention has been drawn to the organo-phosphorous insecticide Dow ET–57 (Etrolene; *O*, *O*-dimethyl–*O*—2, 4, 5—trichlorophenyl phosphorothioate) as a drug that may be administered orally to cattle in the treatment of warble infestation. Reports have appeared from the U.S.A. (e.g. Adkins, 1957; McGregor & Bushland, 1957; Roth & Eddy, 1957; Jones, 1959), the Republic of Ireland (Hatch, 1958, 1959; Kenny & Thornberry, 1958, 1959), Great Britain (Kendall, 1958; Harrison, 1958; Beesley, 1960; Bracewell & Schurr, 1960) and Northern Ireland (Baxter, 1959). In the main these communications have referred to the effects of a single therapeutic dose of Etrolene administered to cattle soon after the end of the season of warble fly activity. Some trials, however, have been conducted in an attempt to establish the optimum time during the autumn or winter for carrying out treatment, whereas in other trials the effects on warble infestation of more than one treatment have been the main consideration.

The purpose of the present contribution is to report on a controlled trial designed to compare the results of a single dose with two doses, to compare early treatment with late treatment and to act as a check on the results obtained in the previous year's trials.

MATERIALS AND METHODS

At seven different farms in Northern Ireland, 357 cattle, mainly Shorthorn and Shorthorn crosses, that had been exposed to the risk of warble infestation during the grazing season of 1959, were available for the trial. They were either store or fattening cattle or heifers intended for breeding. All the animals were identified by numbered ear tags or tattoo marks. The Etrolene was administered in watery suspension as a drench at 100 mg./kg. live weight. At some centres the cattle were weighed, whereas at others an estimated weight was used to calculate the dose. Animals intended for slaughter within 60 days or due to calve in that time were not included. During the winter and spring some of the cattle in the trial were sold and the final number taking part was somewhat reduced.

On farms 1 and 2 the cattle were divided on a basis of age and weight into four groups, one of which was dosed in September, 1959, one group two months later in November, and a third group on both occasions. The fourth group

consisted of the untreated control animals. On four of the other farms the treatment took place in September only, and on farm 5 the cattle were treated in November only.

During March, April, May and June of 1960, at approximately monthly intervals, the cattle were examined by palpation for warbles. The number of warbles present on each animal at each examination was recorded.

RESULTS

The records made on farm 1 are shown in Tables I, II and III.

TABLE I

RESULTS OBTAINED IN 1960 ON FARM 1 AFTER ONE TREATMENT WITH ETROLENE IN SEPTEMBER, 1959

	March		*April*		*May*		*June*	
	T	*UT*	*T*	*UT*	*T*	*UT*	*T*	*UT*
Number of cattle	20	17	20	17	14	13	14	13
Number of warbled cattle	0	0	6	12	7	8	7	9
Number of warbles	0	0	17	111	45	160	30	53

TABLE II

RESULTS OBTAINED IN 1960 ON FARM 1 AFTER ONE TREATMENT WITH ETROLENE IN NOVEMBER, 1959

	March		*April*		*May*		*June*	
	T	*UT*	*T*	*UT*	*T*	*UT*	*T*	*UT*
Number of cattle	14	17	14	17	7	13	7	13
Number of warbled cattle	0	0	4	12	5	8	7	9
Number of warbles	0	0	9	111	29	160	20	53

TABLE III

RESULTS OBTAINED IN 1960 ON FARM 1 AFTER TWO TREATMENTS WITH ETROLENE IN SEPTEMBER AND NOVEMBER, 1959

	March		*April*		*May*		*June*	
	T	*UT*	*T*	*UT*	*T*	*UT*	*T*	*UT*
Number of cattle	15	17	15	17	11	13	10	13
Number of warbled cattle	0	0	2	12	5	8	4	9
Number of warbles	0	0	2	111	14	160	9	53

T = treated cattle. *UT* = untreated cattle.

The results from the other six farms are shown in summary in Table IV. The percentage reduction in warble infestation is calculated on the conventional assumption that groups of both treated and untreated animals of similar age, breed and weight on any particular farm were equally infested before treat-

ment was initiated. Because of this the sums of the numbers of warbles found on the animals provide the basis for comparison after treatment. Some warbles may have been counted more than once but as this applies to treated and untreated animals alike it is considered that the conclusions are not affected by this possibility. In determining the percentage reduction of infestation (% R) the following formula was employed:—

$$\%R = 100 - \left\{ \frac{W.T.}{A.T.} \times \frac{A.UT}{W.UT} \times 100 \right\}$$

where W.T. equals the total number of warbles on the treated animals (A.T.) and W.UT is the number of warbles on the untreated animals (A.UT).

For comparison the results obtained in the 1958–59 trial (Baxter, 1959) on the same farms are also listed in Table IV. In 1958 some 380 animals were involved and the larvicide was given once only, between 18th November and 3rd December.

TABLE IV

SUMMARY OF AVERAGE NUMBERS OF TREATED AND UNTREATED CATTLE ON 7 FARMS, TOTAL NUMBERS OF WARBLES AND THE PERCENTAGE REDUCTION IN WARBLE INFESTATION

Farm	*Treated cattle*		*Untreated cattle*		*Percentage reduction* 1959–60	*Percentage reduction* 1958–59
	Average no. of cattle	*No. of warbles*	*Average no. of cattle*	*No. of warbles*		
1[a]	17	92	15	324	75·2	—
1[b]	10·5	58	15	324	74·5	75·4
1[c]	12·8	25	15	324	91·0	—
2[a]	23	75	19	517	88·0	—
2[b]	20	57	19	517	89·5	66·2[d]
2[c]	22·5	29	19	517	95·3	—
3[a]	10	26	9·5	478	94·9	86·5
4[a]	25	76	26·5	829	90·3	88·4
5[b]	34	264	34	666	69·4	96·8
6[a]	5	5	5	26	80·8	100
7[a]	21	234	17·5	479	59·2	87·2

a = Once treated in September;
b = Once treated in November;
c = Twice treated in September and November;
d = Only 80 mg. Etrolene/kg. live weight was given to the treated animals in this herd in 1958.

DISCUSSION

Within the broad framework of the trial the results do not seem to vary greatly between the years, except on certain farms. The factors responsible for the poor response to treatment on farms 5 and 7 in 1959–60 have not been determined. In the previous year the results were good on both these farms and on both all of the cattle were weighed before treatment. It has been considered (Beesley, 1960) that where the warble infestations are low the reduction in infestation after treatment may not be high. This does not apply to farm 7 as the average infestation of untreated cattle there was 27 warbles. A comparison may be made with farm 2 where the average untreated infestation was also 27 warbles but the reduction was 88·0 to 89·5 per cent.

On farm 5 the average untreated infestation in 1959–60 was 19·4 warbles and the effect of treatment was to reduce the infestation by only 69·4 per cent. On this same farm, in the previous year, the average untreated infestation was even lower, at 14·2 warbles, but the response to treatment was excellent as 96·8 per cent reduction was obtained. Records of weights were kept on farm 5 and the good warble control in 1958–59 was associated with a very slightly better weight gain in the three months following treatment. As would be expected the less marked reduction in warble infestation in 1959–60 was associated with no difference in weight gain between treated and untreated control cattle.

The effect of treating some animals on farms 1 and 2 twice was to give a greater reduction in the number of warble larvae than was obtained by one treatment only. Although the difference is appreciable on farm 1 (about 16 per cent) on farm 2 it is only about 6 per cent. Calculations based on the data given by Kenny & Thornberry (1959) indicate that they got reductions in infestation of 82 per cent with one treatment, 95 per cent with two treatments, and almost 100 per cent with three treatments. As the law of diminishing returns appears to operate, it is doubtful if these relatively minor improvements would compensate for the additional expense involved in administering more than one dose of the preparation.

Under the conditions of this trial it is also evident that the difference in the results between treating in September or in November are so small as to be inconsequential. This is in agreement with Beesley's observations (1960) although it had been considered previously by Hatch (1958) and Baxter (1959) that earlier treatment gave slightly better control of the infestation. Even if this is not so it still appears desirable to initiate systematic larvicidal treatment as soon as possible after the warble flies have ceased their activity so that the larvae may be killed before they cause much tissue damage in their migrations.

SUMMARY

A further controlled trial of Etrolene in the treatment of warble infestation of cattle was conducted in Northern Ireland in 1959–60. The trial was on 357 cattle and comparisons were made between early and late autumn treatment and between the administration of one or two doses of the larvicide. Similar results were obtained from treatment in either September or November. The administration of a second dose increased the reduction in infestation although this enhanced effect was less marked where the response to a single dose was already good.

ACKNOWLEDGEMENTS

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A TECHNIQUE FOR THE ACCURATE MEASUREMENT OF THE RATE OF URINE FLOW OF THE COW FOR RENAL CLEARANCE STUDIES

By R. S. Anderson and E. C. Pickering

Physiology Department, Glasgow University Veterinary School

Accuracy in the measurement of the renal excretion of any substance is largely determined by the efficiency of the urine collection technique. Winton (1956) states that renal clearance measurements under good steady-state conditions involve uncertainties of 5 to 10 per cent, and Smith (1951) ascribes these uncertainties to the difficulty of obtaining complete bladder drainage. Such inaccuracies are exaggerated as the period of collection is shortened. In the cow, a residual bladder volume of 50 ml. is of little importance in relation to a daily output of 14 litres, but represents a large error when the collection period is 15 minutes.

Most investigators of renal function in unanaesthetized cows mention urine collection methods only briefly and say nothing of the difficulties of accurate collection of urine over relatively short periods of time. Sellers and Roepke (1951) do not give details of the catheter which they used during two-hour collection periods. Cunningham, Frederick & Brisson (1955) describe a self-retaining rubber urethal catheter with an inflatable bulb which was employed for the continuous collection of urine over long periods (up to three weeks), and they report that some cows show slight discomfort when first catheterized. Sellers, Pritchard, Weber & Sautter (1958) mention a soft rubber catheter secured to webbing straps for urine collection during clearance periods of 15 to 30 minutes' duration. Poulsen (1957) and Knudsen (1960) describe a self-retaining urethral catheter with an inflatable bulb. De Groot and Aafjes (1960) report inconsistent results when they used urethral catheters in adult cattle, with the additional disadvantage of occasional damage to the urinary tract, and they adopt a special apparatus, described by van Es and Vogt (1959), in which catheterization is avoided. This latter alternative cannot measure continuously the production of urine and would seem to have no application when 15 to 30-minute collection periods are employed.

Modifications of the Foley catheter (Warne, 26 F.G.: 100 ml. bulb capacity) were used in this laboratory in attempts to establish an accurate method of urine collection from conscious cows during 15 to 30-minute periods. In our experience, though satisfactory drainage may be achieved with such a catheter, continuous urine collection may become irregularly intermittent, giving

markedly inconsistent measures of urine formation. This we attribute to occlusion of the perforations of the catheter by folds of bladder mucous membrane.

Two other important difficulties were experienced. First, the negative (sub-atmospheric) intracystic pressure, which is a normal feature of the bovine bladder, results in an inrush of air on catheterization which makes the establishment and maintenance of drainage by siphonage very difficult. Previous workers do not mention this phenomenon. Second, introduction of a catheter into the urethra of an unanaesthetized cow invariably causes struggling, and, once introduced, the inflated retaining cuff resting against the internal urethral orifice causes frequent and sometimes continuous attempts to micturate. Some such attempts can be successful, with loss of urine and sometimes with expulsion of the catheter. The cow also defaecates frequently, soiling the catheter and collection tubing, and shows other manifestations of discomfort. In addition to the inconvenience of this behaviour, such features of an experiment must be considered to be undesirable, because of the effects of pain on the rate of urine flow (Anderson, 1961).

METHOD

The following technique was devised to eliminate the inaccuracies and disadvantages described.

A moulded rubber catheter with a coiled, multiperforate tip is used (a modified "Ramshorn": Vicson 14 E.G.). For introduction this catheter is straightened with a stainless steel stilette and the coiled tip retains the catheter *in situ* when the stilette is withdrawn. As the perforations are present on the inside of the coiled tip they are protected from obstruction. Introduction of the catheter is facilitated by use of a sterile, water-soluble lubricating jelly (Johnson & Johnson "KY" lubricating jelly).

A tightly fitting rubber cuff around the stilette prevents air being sucked into the bladder when the catheter is introduced. The stilette is partly withdrawn once the tip of the catheter is in the bladder and the catheter is clamped before the stilette and cuff are completely removed. The catheter, stilette and cuff are shown in Fig. 1.

With the catheter in place, polythene drainage tubing is attached and the bladder emptied by establishing siphonage. Urine is then collected continuously.

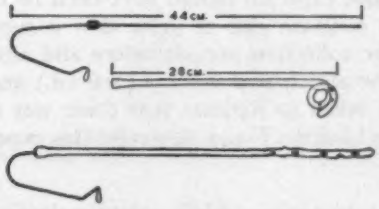

Fig. 1. "Ramshorn" urethral catheter with introducer and cuff.

Discomfort during catheterization and during urine collection, and attempts to micturate and defaecate, are eliminated by prior induction of posterior epidural anaesthesia effected by the injection of 4 ml. of 5 per cent procaine solution (May & Baker "Planocaine") through the sacro-coccygeal space. When an experiment lasts longer than two hours epidural anaesthesia is maintained by additions of 2 ml. 5 per cent procaine solution whenever return of motor function is apparent in the tail and when responses appear on stimulation of the vulva and anus.

RESULTS AND DISCUSSION

A steady flow of urine once the bladder had been emptied was a consistent feature of this technique. Two criteria were used to assess the validity of this method of urine collection: (a) measurement of the volume of fluid returned from the bladder after repeated washing out with a known volume of sterile water or saline, and (b) the constancy of the renal clearance of inulin over wide variations in urine flow.

Bladder washout

Urine was collected continuously for a period of 26 minutes. Then exactly 100 ml. of sterile water or saline was rapidly injected into the bladder through the catheter and the return flow collected immediately in a measuring cylinder. This procedure was carried out three times during the four minutes before the end of a 30-minute period, and each return volume was measured. The difference between the volume of fluid injected and the volume recovered was due to: (1) a previously uncollected "pool" of urine in the bladder, and (2) the urine passing into the bladder from the kidneys in four minutes. In most experiments the difference between the volume of fluid injected and that recovered bore a close relationship to the expected rate of urine formation in the time of the washout.

In an experiment to obtain further evidence of the effectiveness of bladder drainage, urine was collected continuously over a period of 50 minutes with measurements every ten minutes. Five injections of 100 ml. of sterile water were then made into the bladder and the total volume recovered over ten minutes was measured. The mean urine flow prior to washing out was 21·6 ml./min. The total volume recovered during the washout period was 718 ml. Assuming that urine flow had remained constant at 21·6 ml./min., then the recovery volume expected should have been $10 \times 21{\cdot}6 + 500 = 716$ ml. This assumption of a constant rate of urine flow is supported by the good agreement between the collection periods before and after washing out. The correlation between the anticipated volume (716 ml.) and the actual volume (718 ml.) is therefore taken to indicate that there was a negligible residual volume of urine in the bladder. Fig. 2 illustrates this experiment.

Inulin clearance evidence

Glomerular filtration rate remains fairly constant despite wide variations in urine flow (Smith, 1951). If, in the measurement of glomerular filtration rate,

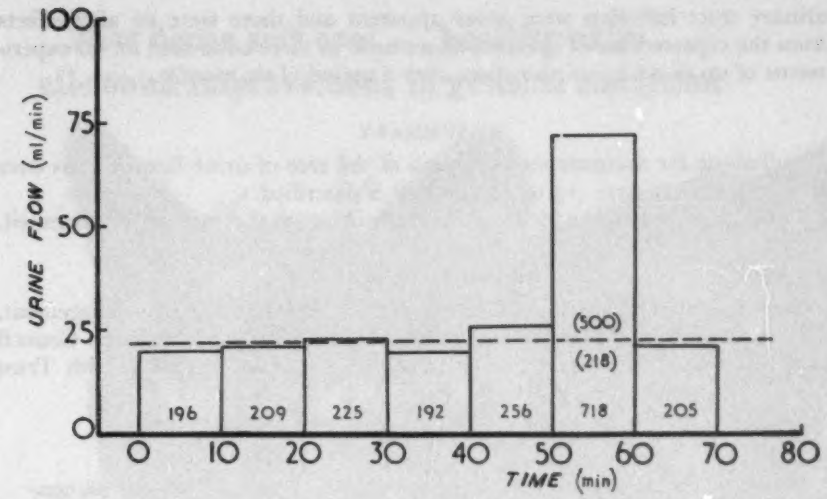

Fig. 2. The effect of bladder washout on urine collection. Five hundred ml. of water were injected into the bladder between times 50 and 60. Figures on histogram are volumes (ml.) collected during each 10-minute period. The broken line indicates a mean urine flow before bladder washout of 21·8 ml./min.

urine collection technique is inefficient, then aberrant values for glomerular filtration rate are to be expected. In the course of repeated measurements of glomerular filtration rate by an inulin clearance technique, there was no evidence of errors in the measurement of inulin clearance which could be attributed to incomplete bladder drainage. Glomerular filtration measurements remained constant within the accepted limits of 5 to 10 per cent (Winton, 1956) despite variations of 200 to 600 per cent in the urine flow. Results of representative experiments on three cows are presented in Table I.

TABLE I

INULIN CLEARANCES (G.F.R.) BEFORE AND DURING DIURESIS

Cow 12916 (1158 *lb.*)		*Cow* 14711 (1202 *lb.*)		*Cow* 14948 (1092 *lb.*)	
Urine flow (*ml./min.*)	*G.F.R.* (*ml./min.*)	*Urine flow* (*ml./min.*)	*G.F.R.* (*ml./min.*)	*Urine flow* (*ml./min.*)	*G.F.R.* (*ml./min.*)
18·8	1265	15·4	918	8·3	958
16·1	1199	7·9	843	50·0	1003
67·5	1274	29·9	941	37·7	924
68·3	1142	32·2	931	28·2	970
61·7	1162	27·7	894	22·9	995
		36·3	864		

Fifteen-minute clearance periods used for three cows at forty to sixty-minute intervals. Diuresis produced by intravenous infusion of hypertonic (molar) KCl solution at 7–9 ml./min.

When the technique described here was used there was no evidence of any side effects from repeated experiments on the same animal. Clinical signs of

urinary tract infection were never apparent and there were no after-effects from the repeated use of epidural anaesthetic in three cows used for 30 experiments of up to six hours' duration, over a period of six months.

SUMMARY

A technique for accurate measurement of the rate of urine flow of cows over a short period of time (15 to 30 minutes) is described.

Some experimental evidence of the efficiency of the method is presented.

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THE PROPHYLACTIC ACTION OF VITAMIN A IN CAECAL COCCIDIOSIS BY PROTECTION OF THE EPITHELIUM

By Edzard Gerriets

Clinical Institute for Poultry Diseases, Humboldt University, Berlin

In addition to its participation in several metabolic processes in the animal body, vitamin A exerts a specific action on the formation, maintenance and regeneration of epithelial tissue. Vitamin A deficiency provokes degenerative processes in the epithelial cells which may affect the function and appearance of epithelial tissues. Keratinization suppresses the natural protective functions of the cell and disturbs the absorption of nutrients. Seifried (1935) has been able to establish that this local susceptibility to infection is reversible. After the administration of vitamin A, keratinized epithelium is regenerated to form functional epithelium and natural resistance is restored. Clinical investigators have found that hypovitaminosis A in both man and animals may be regarded as a predisposing factor for those infections which gain entry through the epithelium.

Coccidia are pronounced epithelial parasites which, during their life cycle, penetrate into the epithelium and multiply. In the last 20 years, considerable research work has advanced our knowledge of the epidemiology and pathogenesis of coccidiosis and has revealed new therapeutic approaches through the introduction of chemotherapeutics having coccidiostatic and coccidiocidal activity. Nevertheless, coccidiosis has continued to increase in incidence and is a source of severe economic loss, particularly to the broiler industry. The transition from extensive to intensive rearing systems has adversely affected the control of the disease. Under extensive management on range it was often difficult to maintain adequate sanitary conditions which would prevent the ingestion of sporulated oocysts, but when economic considerations led to mass production of poultry, even established sanitary precautions were often neglected. This is the situation which at present characterizes the broiler industry and the recognition of this defect has resulted in the increasing use of coccidiostats.

It must also be borne in mind that on range the birds had uncontrolled access to green food containing provitamins. Hawkins (1950) referred to the part played by nutritional factors in the pathogenesis of coccidiosis and reference may be made to the feeding of sour milk and milk products which, though scientifically controversial, has in the past been practised as a prophylactic measure against coccidiosis. The alteration in pH in the intestine undoubtedly

influences the endogenous bacterial flora. Mann (1947) showed that an increase in the carbohydrate content of the ration produced a change in the intestinal flora, and favourably influenced the clinical course of coccidiosis.

It is known that even in conventional doses antibiotics, especially the tetracyclines, exert some, though not a pronounced, influence on the course of coccidiosis, since they selectively inhibit putrefying bacteria, particularly clostridia (Haenel & Gerriets, 1960). Seifried (1935) stated that the lack of bactericidal mucus in hypovitaminosis A results in an increase in the number of saprophytes in the intestine. It may be assumed that disturbance of the microbial balance of the intestine reduces the resistance of the host and renders it vulnerable to infections and infestations such as coccidiosis.

Peak susceptibility to coccidiosis, in the fourth and fifth weeks of life, coincides with feathering, somewhat surprisingly. In addition to the lack of heat insulation at this time, the metabolic processes are also strained to the utmost due to formation of the feathers.

The role of vitamin A in caecal coccidiosis

As early as 1938, Murphy, Hunter and Knandel showed that fish liver oil exerted a favourable action on the course of coccidiosis. Jungherr (1945) pointed out the relationship between vitamin A deficiency and caecal coccidiosis. Davies (1952) observed that in chicks spontaneously affected by coccidiosis the vitamin A content of the organs was lower than in healthy chicks. Schoop, Wagner & Minners (1954) showed experimentally that a deficiency of vitamin A must be regarded as a predisposing factor for coccidiosis in chicks. Wachendorfer (1957) confirmed these results and observed that the excretion of oocysts took place in inverse proportion to the supply of vitamin A. Gylstorff (1959) carried out field observations on large flocks of birds and showed that vitamin A was as capable of influencing the course of coccidiosis as the coccidiostats tested at the same time. Erasmus, Scott & Levine (1960) have successfully demonstrated the correlation between hypovitaminosis A and caecal coccidiosis.

The invasion of the subepithelial layer of the caecal wall by *Eimeria tenella* results in the rupture of capillary vessels in the submucosa and haemorrhage into the lumen of the caecum. This condition is accentuated by a deficiency of the anti-haemorrhagic vitamin K. Further investigations are in progress to determine the degree of any inhibitory influence which coccidiostats may exert on the synthesis of vitamin K by the coliform bacteria. Marthedal & Velling (1961) reviewed the evidence that the haemorrhagic syndrome is associated at least to a certain extent with the administration of sulphaquinoxaline.

If the loss of blood is not fatal it leads to a deterioration in the condition of the animal and favours the secondary development of bacteraemia. The intestinal bacteria including the pathogens can then penetrate unhindered through the mechanically destroyed epithelial cells. This is a process which the cells can no more resist than they can resist the penetration of the dividing forms of the coccidia. Any vitamin A deficiency present prior to infection is

then inevitably accentuated since the extensive damage to the epithelium further hinders vitamin A absorption.

RESULTS

Experiment 1. In a growth test on 100 Rhode Island Red chicks, receiving either natural or the equivalent synthetic vitamins (see Fig. 1), spontaneous caecal coccidiosis appeared during the fifth week of life in all groups. This occurrence presented an opportunity to investigate the effect of vitamin A on the course of the infection. The chicks, therefore, were not treated therapeutically. In the four groups on vitamin A-deficient feed, losses of the order of 76 to 87 per cent occurred, whereas in the four groups receiving vitamin A, losses were drastically reduced (Fig. 2). Groups VI and VIII which were fed vitamin A in mineral-stable form (Rovimix, A; Hoffman-La Roche) showed smaller losses than the groups fed with natural vitamin A. The efficacy of vitamin A on the clinical course of the disease was shown by the significant increase in weight ($P<0{\cdot}001$). In assessing the action of the vitamin A, it should be remembered that only 5,000 i.u. of vitamin A/kg. of feed were supplied; this level approximates to the requirements of the Verband Deutscher Wirtschaftsgeflügelzuchter (Association of German Commercial Poultry Breeders), (1959), for the fortification of compound chick food.

Protocols of Experiments 2–4

Later investigations confirmed the protective action of vitamin A. Rhode Island Red chicks were used throughout and were fed on a standard compounded chick ration containing 500 i.u. of vitamin D_3 (Rovimix) and 5 per cent of torula yeast. The birds were housed in an experimental building having six divisions; it was unheated and the floor had no insulation against damp. Because of the difference in temperature between the warm atmosphere of the shed and the surface of the concrete floor, condensation was always present and the litter became extremely wet. Damp is one of the factors necessary for the sporulation of oocysts in an external environment and therefore coccidiosis regularly appeared in this building. Experimental infection, with its inherent problems, was therefore unnecessary. In all experiments two controls (Groups I and VI) were maintained in the divisions on the corresponding outer sides of the building. The experimental groups (II to V) received vitamin A (water-miscible vitamin A; Hoffmann-La Roche) as a suspension in the drinking water (100,000 i.u./ml.). The finely emulsified vitamin A ensured both easier application and better absorption; it was used because chicks acutely affected by coccidiosis stop feeding but still drink at all stages of the disease as a result of septicaemia and loss of fluid through diarrhoea. Administration of vitamin A started in the second week of life, using amounts shown in Fig. 3. The vitamin A intake of the experimental groups was increased weekly during the seven weeks of the experiment, by an amount equal to the initial dose.

Experiment 2. Coccidiosis appeared as anticipated in all the experimental groups during the fourth week. Although the mortality rate in the control

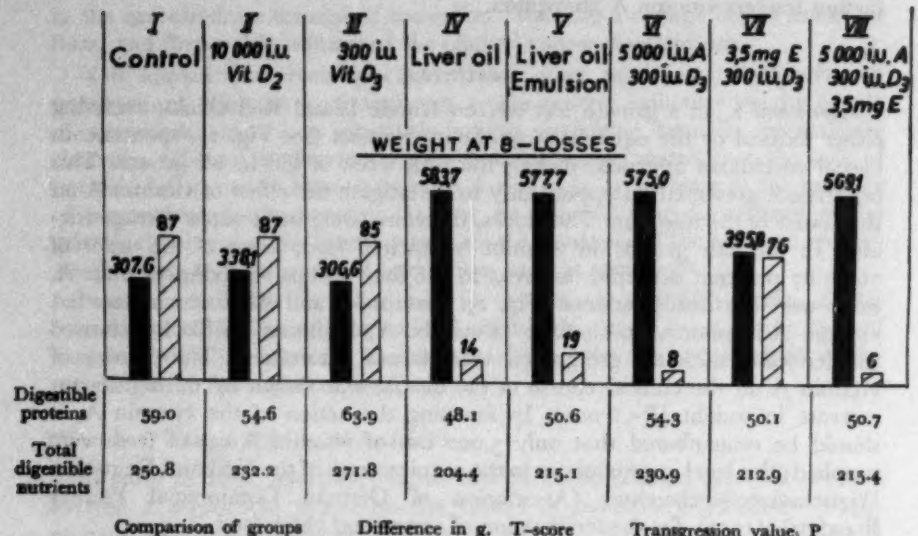


Fig. 1. Experiment 1.

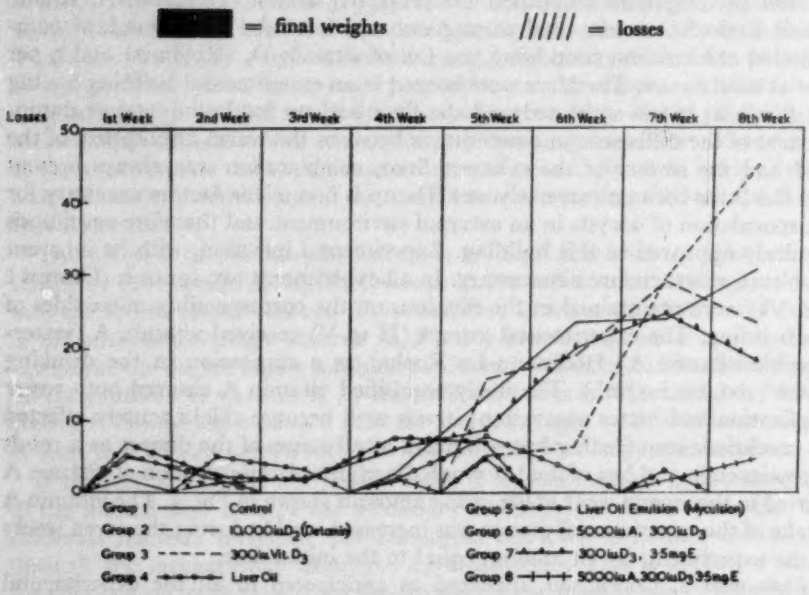


Fig. 2. Weekly losses in experiment 1.

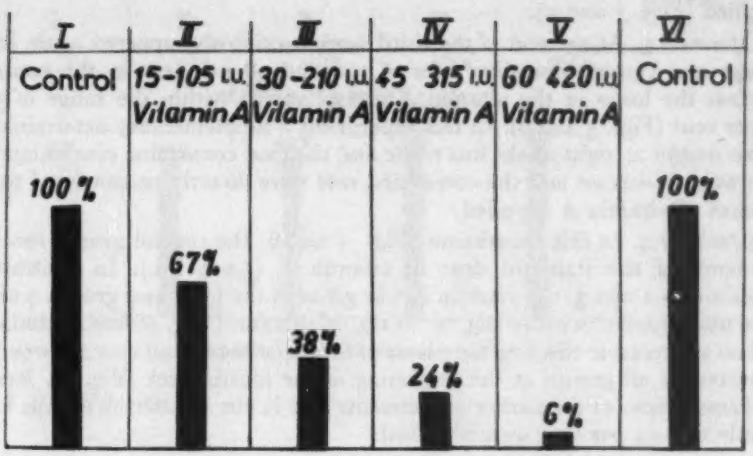


Fig. 3. Losses in experiment 2.

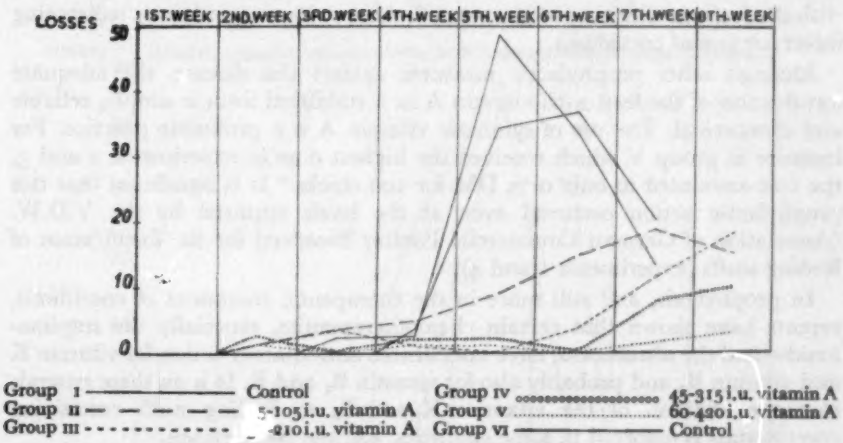


Fig. 4. Weekly losses in experiment 2.

groups was 100 per cent, the losses in the four vitamin A groups were 64, 38, 34 and 6 per cent and decreased in direct proportion to the amount of vitamin A supplied (Figs. 3 and 4).

Experiment 3. At the end of the third week coccidiosis appeared again in all groups and caused mortality rates of 100 and 98 per cent in the controls, whereas the losses in the vitamin A groups varied within the range of 5 to 11 per cent (Figs. 5 and 6). In this experiment a supplementary determination of the weight at eight weeks was made and the food conversion rate estimated. The weight increase and the conversion rate were directly proportional to the amount of vitamin A supplied.

Experiment 4. In this experiment (Figs. 7 and 8) the control groups received one-tenth of the standard dose of vitamin A (5,000 i.u.). In contrast to experiments 2 and 3, the vitamin A was given in the feed, and groups 3 and 4 were also supplied with 10 mg. or 20 mg. of vitamin C/kg. of feed. Vitamin C had no appreciable effect on the course of the experiment, and caecal coccidiosis appeared in all groups at the beginning of the fourth week (Fig. 8). Results confirmed those of the earlier experiments and in the vitamin A groups losses of only 6 to 11 per cent were observed.

DISCUSSION

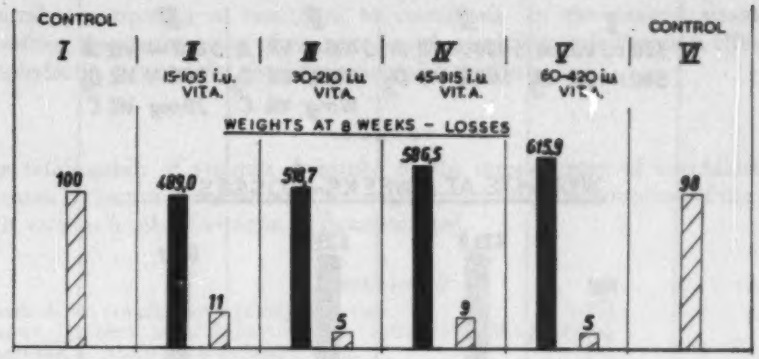
Experiments carried out on 2,400 chicks have shown that vitamin A beneficially influences the appearance and clinical course of caecal coccidiosis. This effect, first observed in a spontaneous outbreak of coccidiosis (experiment 1), was confirmed in three experimental tests (experiments 2 to 4). This is in agreement with the findings of Schoop *et al.* (1954) that hypovitaminosis A is a predisposing factor for caecal coccidiosis.

Amongst other prophylactic measures against this disease, the adequate fortification of the feed with vitamin A in a stabilized form is simple, reliable and economical. The use of synthetic vitamin A is a profitable practice. For instance in group V which received the highest dose in experiments 2 and 3, the cost amounted to only 0·75 DM for 100 chicks.* It is significant that this prophylactic action occurred even at the levels required by the V.D.W. (Association of German Commercial Poultry Breeders) for the fortification of feeding stuffs (experiments 1 and 4).

In prophylaxis, and still more in the therapeutic treatment of coccidiosis, reports have shown that certain chemotherapeutics, especially the sulphonamides and the nitrofurans, have undesirable anti-vitamin action for vitamin K and vitamin B_6 and probably also for vitamin B_2 and E. It is on these grounds that the addition of the vitamins K and B_2 to feeding stuffs containing coccidiostats is enforced in some countries, e.g. the Netherlands.

The undesirable side effects of coccidiostats, coupled with the fact that their prophylactic action is not consistently satisfactory, emphasizes the value of

* In England the cost of treating 100 chicks for five weeks at approximately 250 i.u./lb. live weight per day would be about 1s. 6d.

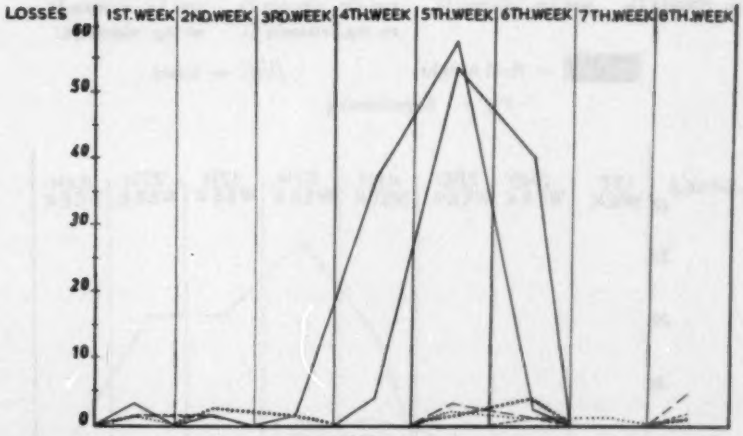


Conversion figures for 100 g. increase in weight

Digestible proteins	52·8	50·3	44·5	48·6
Total digestible nutrients	224·3	213·6	202·0	206·8

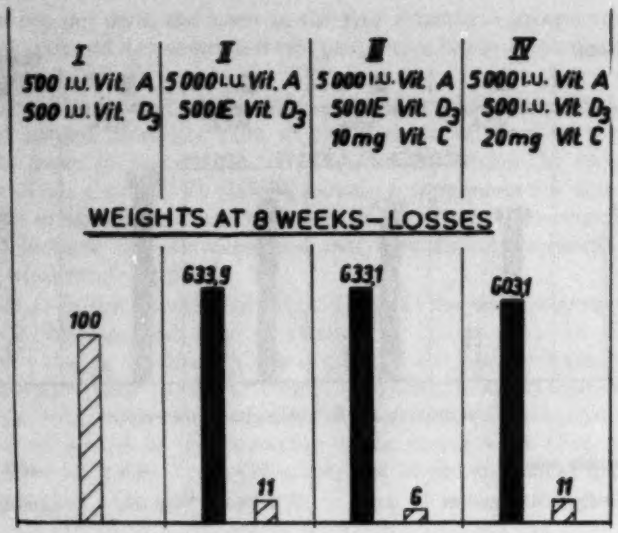
■ = final weights , ////// = losses

Fig. 5. Experiment 3.



Group I ———— Control
Group II — — — — 15-105 i.u. of vitamin A
Group III - - - - - - - - 30-210 i.u. of vitamin A
Group IV ..... 45-315 i.u. of vitamin A
Group V ..... 60-420 i.u. of vitamin A
Group VI ———— Control

Fig. 6. Weekly losses in experiment 3.



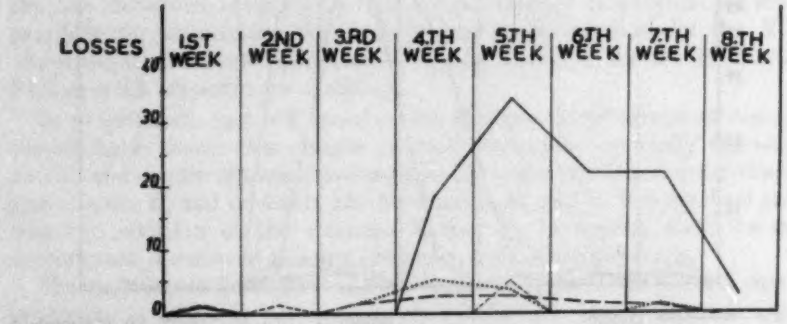
Food conversion figures for 100 g. increase in weight

Digestible proteins	48·2	49·0	49·1
Total digestible nutrients	205·1	208·3	208·6

I	II	III	
500 i.u. vitamin A 500 i.u. vitamin D_3	5,000 i.u. vitamin A 500 i.u. vitamin D_3	5,000 i.u. vitamin A 500 i'u. vitamin D_3 10 mg. vitamin C	5,000 i.u. vitamin A 500 i.u. vitamin D_3 20 mg. vitamin C

■ = final weights ////// = losses

Fig. 7. Experiment 4.



Group I ———— 500 i.u. of A, 500 i.u. of D_3 Group II — — — — 5,000 i.u. of A, 500 i.u. of D_3
Group III - - - - - 5,000 i.u. of A, 500 of D_3, 10 mg. C Group IV ..... 5,000 i.u. of A, 500 i.u. of D_3, 20 mg. C

Fig. 8. Weekly losses in experiment 4.

vitamin A which, in addition to its essential functions in metabolism, promotes natural development of resistance to coccidiosis. In the present situation, therefore, the combination of vitamin A with coccidiostats is the most effective prophylactic measure for the prevention of coccidiosis.

SUMMARY

The relationship of vitamin A intake to the development of coccidiosis in chickens is discussed and the effect of experimental supplementation of the diet with various levels of vitamin A demonstrated.

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(*Received for publication*, 8 *August*, 1961)

BOOK REVIEWS

A GENERAL ACCOUNT OF DISEASE CONTROL IN PIGS

Diseases of the Pig. By D. J. Anthony and E. F. Lewis. Fifth edition. London: Baillière, Tindall and Cox. Price 35s.

The late E. F. Lewis joined D. J. Anthony as co-author for the fifth edition of this book. A chapter on diseases of newly born and young pigs has been added. The technique of tuberculin testing and posological details of the commoner theraupetic preparations now appear as appendices. The general layout is unaltered and the first five chapters covering husbandry continue to be the more satisfactory section of the book. Some of the remaining chapters show evidence of revision and additions have been made to the lists of references which appear at the end of each chapter. It would seem from reading this and previous editions that great emphasis is laid on the need to be comprehensive and this leads to the inclusion of a variety of conditions rarely if ever seen in life. This, together with the limitation of space and a discursive style of writing, inevitably result in information being presented in very general terms. The book remains, of course, the only British text available but as it stands it will probably be most useful to the pig farmer and breeder and to those requiring information on a wide range of topics associated with pig breeding. Those readers who feel the need for more detailed and fundamental information will be assisted by the lists of references, many of which are to papers in readily obtainable journals.

The quality of publication is well up to the high standard to which one has become accustomed from Baillière, Tindall and Cox.

C. S. Grunsell

A STUDY OF THE MANAGEMENT OF FARMS

Farm Management. By James Wyllie. Third edition. London: E. & F. N. Spon Ltd. Price 40s.

In this new edition of a well-known book additional attention has been given to the analytical method of studying farm management and also the book has been thoroughly revised to bring it into line with some of the more recent developments in agricultural practice.

There are chapters on farm economics and general farm management and in addition chapters on management in relation to individual enterprises such as milk production, beef production, pig husbandry, etc. Mr. Wyllie in writing this book has drawn on his long experience of agricultural economics and farming; he reaches a number of interesting conclusions and gives much common-sense advice. Among the many interesting aspects discussed is the complicated nature of farm management and the wide gap which exists between economic theory and its application to the individual farm. The chapters dealing with the management of individual enterprises are of necessity superficial in a work of this nature and although certain aspects of animal

management are discussed at some length the subjects are not dealt with comprehensively. Those interested in the details of animal management would need to consult more comprehensive works.

This book would certainly be of interest to agricultural students and to the townsman thinking of becoming a farmer as it contains much information and general advice on farming.

J. S. S. INGLIS

VACCINATION AGAINST TUBERCULOSIS

Fundamentals and Possibilities in Anti-Tuberculosis Vaccination. By RICHARD PRIGGE and GUNTHER HEYMANN. Translated by H. CHANDLER ELLIOTT. Canada: University of Toronto Press. Price 40s.

This book is obviously intended for the medical profession as vaccination against bovine tuberculosis is no longer a tenable proposition owing to the success of eradication schemes.

It is an interesting book and deserves a place in the library of all who are concerned with the prevention of tuberculosis. There are chapters on resistance, immunity and allergy, and also a chapter on the relationship between allergy and immunity. These are well worth studying.

There is a chapter on the chemistry of the tubercle bacillus which brings one's knowledge on this subject up to date.

Methods of vaccination are discussed and one has the feeling that the authors are biased against the use of B.C.G. vaccine and that undue weight is given to the case against its use. Few people today believe that there is any danger associated with the use of B.C.G. vaccine and if the evidence in favour of its use is not completely satisfactory, it would be interesting if the authors had set out the type of experiment which would be convincing to them and at the same time be a practical proposition.

Despite this criticism the book is well worth reading and the case against the use of B.C.G. vaccine is, on the whole, convincing.

H. G. LAMONT

CORRESPONDENCE

AFRICAN SWINE FEVER

Sir,

The suggestions made by Doyle in a footnote to his article on African Swine Fever, published in the June issue of your journal (Vol. 117, p.229) under the heading of African Pig Disease, that this latter name be used to refer to a specific disease of swine originally described from Africa and now present in the Iberian Peninsula, is unacceptable on many grounds. That *The Times* should refer to African Swine Fever as African Pig Disease is journalese and no good reason is advanced as to why this heading to a column should be perpetuated.

Those who use the appellation African Swine Fever in their conversation almost daily are conscious of the inaccuracy of that name for this condition of pigs first described from Africa, but little would be gained by accepting Doyle's 'appropriate' name. It would be even more misleading than the present name since there are many diseases of pigs in Africa, both recognized and as yet unrecognized.

It is important, however, that now it is firmly established that the disease is distinct from the classical Swine Fever of Europe and Hog Cholera of America (another misnomer) it should have a distinctive name. Some attempt has been made to do so by Portuguese and Spanish authors who refer to the condition as Montgomery's disease of pigs, for obvious reasons. Fortunately the veterinary profession is not so prone as its sister medical profession to label a syndrome with the name of the person who first described it and this attitude should be encouraged; this therefore rules out the term Montgomery's disease.

Before any change is made it would be interesting first to see what name has been used in the forthcoming publication edited by W. A. Pool, entitled "Nomenclature of Diseases of Animals". While it is already conceded that no disease should include in its name any geographical indication in these days of rapid and frequent intercontinental movement, and that the name should give some indication of its host specificity, aetiological group and histopathology, it would be premature even with African Swine Fever, to suggest a new name on this basis. Rather should discussion await the publication referred to.

Yours faithfully,

W. G. BEATON.

Interafrican Bureau for Animal Health (C.C.T.A),
Muguga.,
P.O. Kikuyu,
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Studies on Disinfection of Eggs and Incubators. V. The Toxicity of Formaldehyde to the Developing Embryo.

D. W. Brocklesby, S. F. Barnett and G. R. Scott
East African Veterinary Research Organization, Muguga, Kenya.
Morbidity and Mortality Rates in East Coast Fever (*Theileria parva infection*) and their Application to Drug Screening Procedures.

F. C. Pinto
Government Analyst's Laboratory, Ceylon.
Serological Identification of Ox, Buffalo, Goat and Deer Flesh.

I. D. B. Newsam
Veterinary Investigation Centre, Ministry of Agriculture, Fisheries and Food, Cambridge.
The Cellular Picture of Vaginal Mucus from Normal Heifers.

T. Grahame
Dept. of Veterinary Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh.
The Arterial Circulation of the Kidney of a Young Elephant (*Elephas indicus*).

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